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Agricultural Research Institute, Pusa

Syngamus laryngeus in Cattle and Buffaloes in India

BY

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Director and First Bacteriologist,
Imperial Bacteriological Laboratory, Muktesar;

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Syngamus laryngeus in Cattle and Buffaloes in India.

[*Received for publication on 9th August, 1919.*]

THE only published account of a *Syngamus* parasite occurring in cattle appears to be that by Railliet in the *Comptes Rendus de la Société de Biologie* for 1899, p. 174.

In the records of this laboratory there is evidence that the same or a similar parasite was found in cattle at Muktesar in 1897, but no account of it was published.

In 1916 it was observed independently by one of us (A. W. S.), and again in 1918 (A. L. S.)

This note is based upon the examination of worms obtained from animals, hill bulls and buffaloes, used for the production of rinderpest virus, and also from plains cattle of various ages and of both sexes killed in the slaughter-house at Bareilly.

Nearly two hundred buffaloes, one hundred plains cattle, and nearly five hundred hill bulls have been examined. Parasites were found in about 13 per cent. of the buffaloes and hill bulls, and about 15 per cent. of the plains cattle. The maximum number found in any one animal was 17 in the case of the hill bulls, 24 in the case of the buffaloes, and 16 in the case of plains cattle. As a rule, however, only one or two were found.

With few exceptions, all were found attached to some part of the larynx. They have been found on the inner surface of the arytenoid cartilages, the bezel of the cricoid near its anterior edge, the vocal cords, the lateral ventricles and epiglottis (Fig. 1).

The observation of 1916 is not taken into consideration in the above statement regarding the usual situation of the parasite.

In that case the subject was a hill bull which had been used for some time for the preparation of anti-hæmorrhagic septicæmia serum.

The animal had been coughing frequently and had had a poor appetite for some days when it was noticed that particles of food were expelled from the nostrils during the act of coughing. This had continued for a few days when the animal died unexpectedly during the night.

Apart from extreme debility, which was probably due to the repeated injections and bleedings which the animal had had to undergo as a serum-maker, the general post-mortem yielded negative results.

The mucous membrane of the pharynx was, however, congested, and there were found adhering to it and to the back of the soft palate some ten or twelve pairs of worms. A few were also found attached to the mucous membrane of the larynx. It is probable that the parasites present in the pharynx set up some degree of irritation which was responsible for the symptoms observed during life.

As already mentioned, the parasite has been found in bulls coming from the Kumaun and Garhwal hills, and in buffaloes and cattle from the Bareilly District in the plains. That buffaloes in the plains are actually infected with the parasite independently of any infection they may possibly have contracted at Muktesar, is proved by the fact that a watch was kept at the Branch Laboratory at Bareilly during the cold weather among buffaloes purchased locally, and a number of specimens were found.

That the bulls did not acquire the infection from buffaloes in Muktesar is indicated by the fact that the percentage of infected animals is about the same in both species, and by the fact that bulls coming from the hills are kept in separate kraals from the buffaloes until they are brought into the sheds. As virus producers their life in the sheds is too short to permit of their becoming infected in such numbers.

These points establish the facts that both cattle and buffaloes are hosts of the parasites, and that the infection is about as common in the plains (at least so far as the Bareilly District is concerned) as in the hills.

Age appears to play no part in determining the infection as the parasite has been found in animals of all ages from 1 to 12 years old.

Morphology. The majority of the parasites are bright red in colour, but occasionally one or other or even both of a pair may be quite white. This is no doubt due to their having ceased to suck blood for some reason or other.

Railliet gives the measurements of the worms found in cattle in Annam as follows :—Males from 3 to 3·375 millimetres in length by 360 to 375 microns in thickness ; females 8·75 to 9·8 millimetres in length by 550 to 570 microns in thickness.

The specimens which we have had for examination have given the following range of size :—Males 3 to 5 millimetres in length by 340 to 375 microns in thickness ; females 7 to 20 millimetres in length by 450 to 675 microns in thickness (Fig. 2).

From this it will be seen that many of the worms examined by us were at least twice as large as those examined by Railliet.

Male worm. The male is approximately constant in thickness throughout its length (Fig. 3), but shews a slight abrupt constriction at the line of junction between the body and the caudal bursa (Fig. 4).

In no instance have males and females been found unattached to each other.

The cuticle does not shew any distinct cross striation in the fresh condition, but it can be made out obscurely in preserved specimens, and it is distinctly visible in specimens that are partially dry.

The anterior end is squarely truncated and shows the circular mouth.

At a distance of about 0·75 mm. from the anterior end the cuticle shews a small blunt tubercle on each side of the body (Fig. 5).

The caudal pouch is somewhat poorly developed as regards its size (Figs. 3 & 4).

Alimentary tract. The mouth is circular and is surrounded by a thin transparent membranous lip. From the examination of longitudinal sections it appears that this lip is composed of two layers between which there is a space which forms a closed canal round the orifice (Fig. 6. A). A similar canal encircles the parasite just posterior to this.

The buccal capsule is almost hemispherical in shape and is composed of thick chitinous material (Fig. 6. B).

At the bottom of the capsule, round the opening of the œsophagus, there are eight small elliptical holes (Fig. 6. C). Immediately external to these there is a ring of eight chitinous points projecting forwards into the mouth cavity (Fig. 6. D). From the base of each of these there arises a thick rib which runs up on the inner surface of the capsule.

It is a somewhat peculiar fact that only six of these ribs reach the margin of the capsule. One pair which are opposite to each other fade out when about two-thirds of the distance has been traversed (Figs. 7 & 8). In this respect the parasite is possibly different from that referred to by Railliet who describes the whole of the ribs as reaching the margin. The margin of the capsule is thus divided into six segments only, of which two are twice as large as the remainder. Examination of the capsule under a high magnification shews that throughout its extent it is beset with minute perforations set closely together (Figs. 6 & 9).

The œsophagus which has the usual club-like shape is particularly well developed. The intestine runs a practically straight course to the hinder end of the body.

The anterior part of the intestine is expanded in such a manner as to almost merit being termed a stomach. In fresh preparations this "stomach" is shaped like a peg top, the bulbous end being the anterior one (Fig. 10). In fixed specimens it is generally telescoped.

The minute anatomy of the intestine is the same throughout its length. Outwardly there is a layer of large cells whose margins are difficult to make out. These cells not infrequently have a frothy appearance as if crowded with fat droplets. On the inner face of these there is a thin layer of homogeneous material which stains intensely with eosin. The inner surface of this layer is covered with short rod-like structures set at right angles to it and packed closely together (Fig. 11).

When seen from the surface this layer has a mosaic-like appearance. Posteriorly the intestine opens into a funnel-shaped cloaca (Fig. 12).

Genital system. The striking fact in connection with the genital system of the male parasite is the complete absence of any spicules. The entire system appears to be represented by a single tube which is closed anteriorly and opens posteriorly into the cloaca. This tube is about three-quarters the length of the whole worm, but is invariably coiled so as to occupy a position in the hinder half of the parasite only (Figs. 3, 4).

Structurally the tube comprises two distinct parts which are approximately equal in length. The anterior half of the tube is the germinal portion in which the spermatozoa are developed (Fig. 13. A). Here the tube is packed with small rounded cells with compact nuclei. These cells do not appear to be provided with any locomotor apparatus. The hinder portion of the tube shews a distinct lumen bounded by large cells. In paraffin sections the spermatic fluid can not infrequently be found in the lumen of this portion of the tube (Fig. 13. B).

The tube enters the cloaca dorsally to the intestine.* The cloaca has a comparatively narrow outlet but no accurate measurements could be made as some degree of distortion takes place when sections are cut, no matter what method is adopted, and the orifice of the cloaca cannot be seen distinctly except in sections.

The caudal bursa (Figs. 3, 4 & 14), which may be considered as part of the genital apparatus, is peculiar in many respects. It is smaller than is usually found in the parasitic round worms, but is very much thicker and is in fact quite fleshy. Its smallness is, however, compensated for as will be seen later. Its small size, thickness, and the closeness with which it adheres to the female make it difficult to study the pouch in detail. A complete idea of the details of the pouch can only be arrived at as the result of the examination of a number of specimens prepared in different ways, sections cut in paraffin, by freezing and teased preparations.

The pouch may be described as more or less saddle-shaped, the panels of the saddle passing over the sides of the female. The lateral lobes of the pouch are connected anteriorly by a flap which is thicker than any other part. This anterior part is recurved, this serving to anchor it firmly to the anterior margin of the flattened boss on the body of the female in which the vulva opens (Fig. 15. A).

Posteriorly the lateral lobes of the bursa are directly connected by a moderately thin flap. This is to some extent recurved in fresh specimens but not to so marked an extent as the anterior lobe (Fig. 15. B).

This arrangement alone would account for a considerable amount of difficulty in detaching the parasites from each other, but the union is rendered far more intimate by the fact that the whole of the inner surface of the pouch, with the possible exception of one small area, is closely beset with small crypts (Fig. 16) into each of which is fitted a small pointed projection developed from a special layer on the surface of the female over the whole area included within the grasp of the pouch (Fig. 17).

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* It is assumed that the ventral surface of the male is directed towards the anterior end of the female.

Sections of the male worm shew that throughout its extent the pouch is spongy in structure. It comprises an inner and an outer dense layer and between these a loose-textured connective tissue, the main strands of which pass straight from one dense layer to the other (Figs. 12, 17 & 18).

The supporting rays of the pouch are poorly developed and difficult to see in detail in intact parasites (Fig. 3).

The best pictures of them have been obtained in serial sections cut in paraffin. From these it is seen that there are two main trunks. The anterior one gives rise to three broad rays with rounded ends which lie in close contact with each other (Fig. 18). From the posterior trunk two rays of a similar nature take origin.

Other organs. In the anterior part of the body there are two large blind pouches which appear to open by a small orifice common to both through the cuticle on the posterior surface of the body at a point opposite the junction of the anterior and middle thirds of the œsophagus (Fig.19).

These pouches become wider as they pass backwards and terminate about the middle point of the body. Projecting from the blind end of each there is a little appendix-like prolongation. The nature and function of these has not been determined. Possibly they are the homologues of the cervical glands described by Looss in *Anchylostoma duodenale.*

In the body cavity, and apparently unattached in any way, there are invariably four rounded bodies (Figs. 3 & 4), measuring 100 to 120 microns in diameter, which are largely composed of closely felted filaments each of which shews a close succession of rounded thickenings, thus producing an appearance resembling a string of beads.

In sections of these bodies two distinct zones can be made out. The outer zone is composed of the filaments described, and a central area which stains more intensely, is finely granular, and in which a few nuclei can be detected. The nature and function of these is also unknown.

Female worm. The female worm of which the measurements have already been given, attains its maximum thickness in the neighbourhood of the vulva. It tapers slightly towards the head and tapers continuously towards the tail where it terminates in a subacute point.

What has been said regarding the presence of striations on the cuticle of the male holds good for the female.

The mouth and alimentary tract of the female are, except in the matter of size, the counterpart of the corresponding parts of the male.

The anus in the female is sub-terminal, at a distance of about 300 microns from the tip of the tail, and takes the form of a transverse slit.

If the piece of cuticle containing the vulva be dissected away from the rest of the parasite and spread out flat, a more or less circular area bounded by a distinct line can be seen under a low magnification. This has a slightly granular appearance and is the area covered by the bursa of the male (Fig. 21).

In serial longitudinal sections it is found that this line circumscribes a flattened boss which is slightly undercut to give anchorage to the recurved flaps of the caudal pouch of the male. This undercutting is most marked in worms that have been sectioned while still attached to each other. If the worms be separated before fixing, the boss is scarcely salient at all.

The vulva takes the form of a transverse opening and is not furnished with any protective appendage or prominent lips. It opens somewhat posteriorly to the central point of the boss. As already mentioned, the boss is covered with a specialized layer the surface of which shews immense numbers of little pointed processes for insertion into the crypts on the under-surface of the bursa of the male (Figs. 17 & 22). It appears to be probable that this specialized layer is in reality in the nature of a cement substance which is drawn up into the crypts of the pouch by suction. It is difficult to suppose that actual outgrowths of the cuticle could be fitted so accurately into so many minute crypts of varying size. It is probable that there is one area of cuticle, extending backwards from the vulva, which is not in intimate union with the under-surface of the caudal pouch.

Sections have been examined in which eggs had reached the vagina and even the vulva, and were on the point of being extruded. In view of the facts that the vagina is directed backwards and that the vulva opens posteriorly to the centre of the area covered by the pouch, it seems to be reasonable to suppose that the eggs are passed out under the posterior flap of the pouch. That there must be some channel of this sort is indicated by the fact that there must be some means of escape for eggs and for the waste material passing from the alimentary canal of the male.

The less complete anchorage of the posterior lobe of the pouch also suggests that this may be the case.

It would appear to be a very wasteful procedure on the part of Nature if, as has been suggested, the eggs can only escape as a result of rupture or disintegration of the body of the female. This would be tantamount to the death of the parent worm. A very large proportion of the eggs liberated would in all probability not be viable.

That the view put forward is correct is borne out by the fact that eggs have been observed on a number of occasions in the act of escaping from beneath the caudal pouch of the male. Living worms were obtained and placed in 0·4 per cent. salt solution. Under a dissecting microscope the eggs were observed to be ejected with some force from under the posterior flap of the caudal pouch, in some cases at intervals of a few seconds and in others at very long intervals.

Genital organs of the female. The ovaries and uteri are twisted round themselves and round each other in so complicated a manner that it has been found impossible to disentangle both branches of the system, for the purpose of measurement, without damage. In many instances it has been found possible to ascertain the length of one branch by sacrificing the other. It has been found that the length varies from two to two and a half times the total length of the worm.

From the examination of serial longitudinal sections it appears that the vagina, which is very short (Fig. 21), opens into a funnel-shaped vulva which is compressed dorsi-ventrally. In many paraffin sections the minute cells described as being present in the duct portion of the male genital tube could be found crowded into the funnel-like opening and passing down into the uteri (Figs. 23 & 24).

From the vagina, which is about 150 microns in length and which is provided with very thick walls, two uteri take origin. These are thin-walled tubes which vary greatly in diameter, depending upon the number of eggs contained in them. They measure about 5 millimetres in length in a fully grown parasite. Each branch of the uterus is continued as a long slender ovary measuring 40 to 50 millimetres in length. The ovaries terminate blindly. The blind pouches, described as being present in the anterior part of the male, and the four ball-like structures are present in the female worms, and are proportionately larger.

Eggs. In the distal portions of the ovaries the first evidence of egg formation is the appearance of segmentation in the ovarial contents. In sections stained with hæmatein and eosin the segments are found to be crowded with granules staining intensely with the hæmatein. Further down the tubes, where the eggs have assumed the oval shape, these granules are found disposed at the surface and closely touching each other. In the next stage of development the granules have entirely disappeared and the shells of the eggs have made their appearance (Fig. 25). It appears to be probable that the shells are made from these granules.

The whole process very closely resembles that observed in the stages of development of the oocyst from the macrogamete of Coccidium.

The most mature eggs found in the uteri are in the morula stage comprising eight cells, but eggs were found in the mucus on the surface of the membrane of the larynx in which only two cells could be seen (Fig. 26).

The mature eggs measure about 80 microns in length by 40 in width No evidence of an operculum can be found.

Pathogenic effects. With the exception of the special case cited above no disturbance of health has been noted as being caused by the parasite.

Figures 27 and 28 shew respectively longitudinal and transverse sections of the mouth parts of parasites still attached to the mucous membrane.

In Figure 26 it is seen that a " button " of the membrane is drawn into the mouth, and no doubt the chitinous points or lancets set round the opening of the œsophagus puncture the membrane and thus enable the parasite to withdraw blood.

Technique. It has not been found possible to obtain uniformly satisfactory results by any of the methods of preparing sections that have been described, but good results have been obtained at different times with all of them.

It was found to be essential for the penetration of the paraffin to make an opening in the integument as it offered a great resistance. This was generally effected by cutting off a portion of the worm that was not required for examination. It was frequently found to be of great assistance in cutting sections of the worms to adopt the plan described by Looss of painting the surface of the paraffin block with a thin solution of celloidin between each cut.

The chitinous parts of the mouth presented great difficulty when cutting specimens embedded in paraffin, as they shewed a great tendency to crack.

To overcome the difficulty of small parts of the sections becoming detached from the slides while the processes of paraffin extraction and staining were being carried out, the plan was on some occasions adopted of staining the specimens without removing the paraffin. It was found that this could be done successfully *provided the paraffin wax was not actually melted when the sections were placed upon the surface of warm water* prior to placing them upon the slides. The processes of staining and dehydration were carried out in the usual way and the xylol used for clearing simultaneously removed the paraffin and left the specimens ready for mounting in balsam. The painting of the surface of the block with celloidin did not interfere with this method as the celloidin was removed by the alcohol used for dehydration.

On a large number of occasions, sections, quite satisfactory for some purposes, were cut with the freezing microtome. These sections were washed off the razor either singly or two or three at a time by ejecting a gentle stream of water from a pipette on to the surface of the blade and draining it with the sections either on to a slide or into a watch glass.

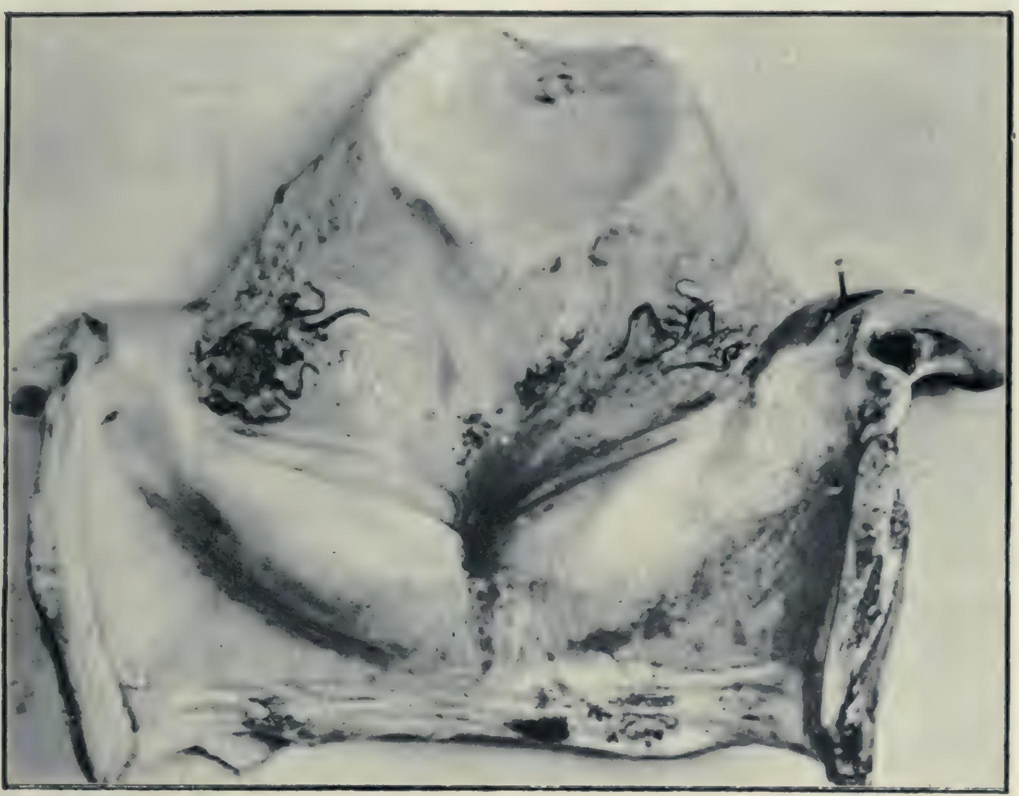

Fig. 1. A larynx laid open by cutting through the bezel of the cricoid. Shews a group of worms attached to the ary-epiglottic fold on each side.

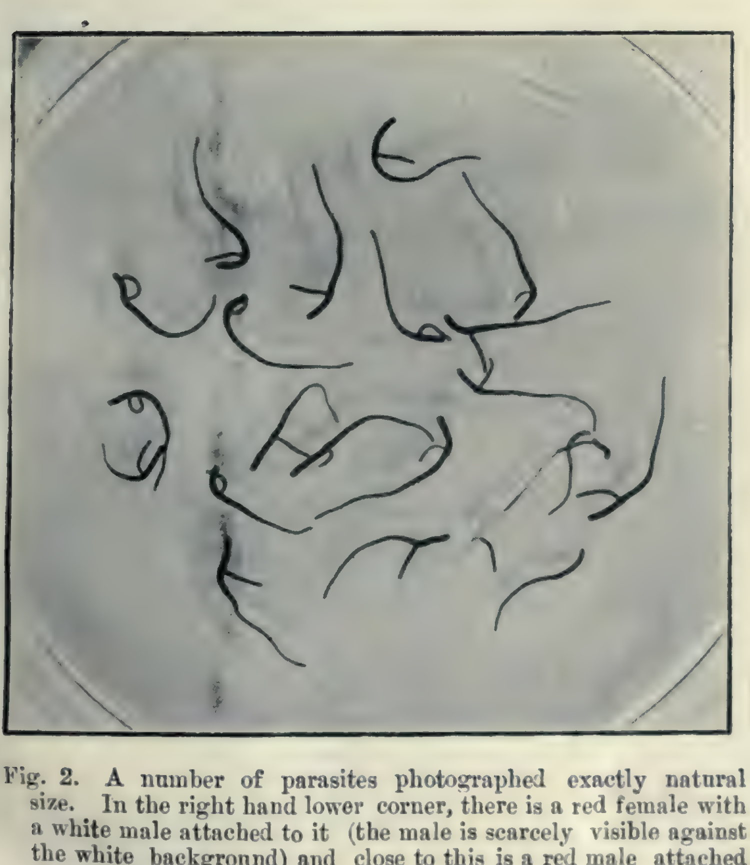

Fig. 2. A number of parasites photographed exactly natural size. In the right hand lower corner, there is a red female with a white male attached to it (the male is scarcely visible against the white background) and close to this is a red male attached to a white female.

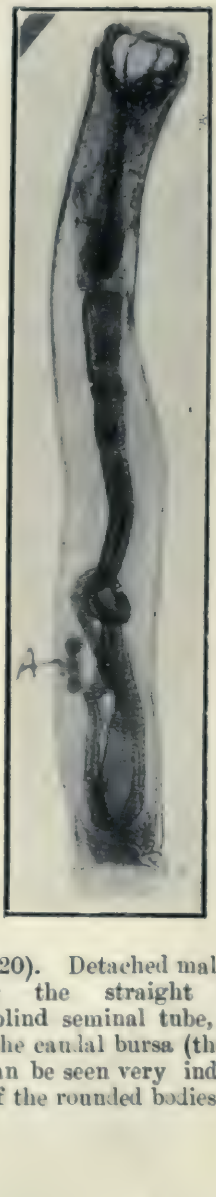

Fig. 3 (× 20). Detached male parasite shewing the straight intestine, single blind seminal tube, the small size of the caudal bursa (the rays of which can be seen very indistinctly). Three of the rounded bodies at A.

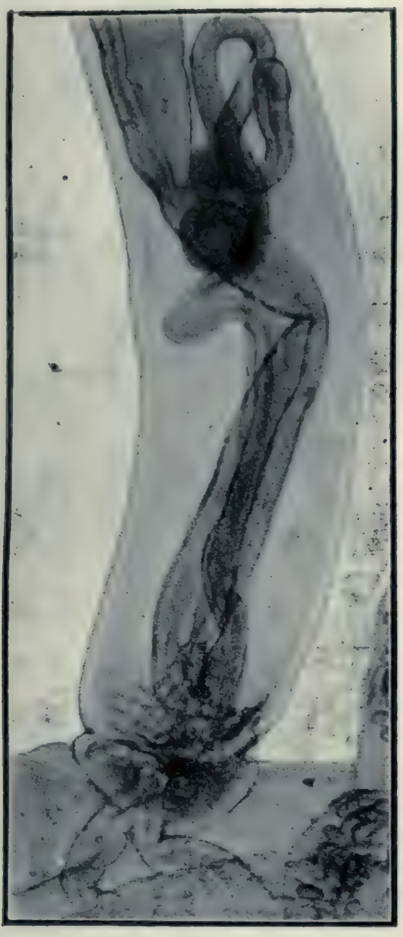

Fig. 4. The junction of the male and female shewing the slight constriction at the junction of the caudal bursa with the body, the single blind seminal tube, the small caudal bursa, two of the rounded bodies.

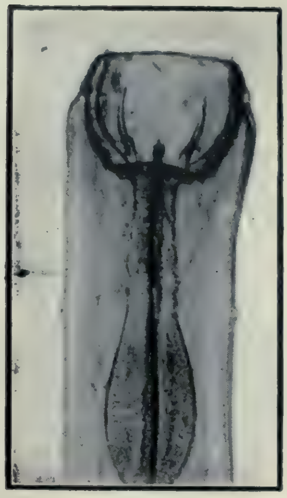

Fig. 5. Anterior end of the male shewing two blunt tubercles on either side of the body.

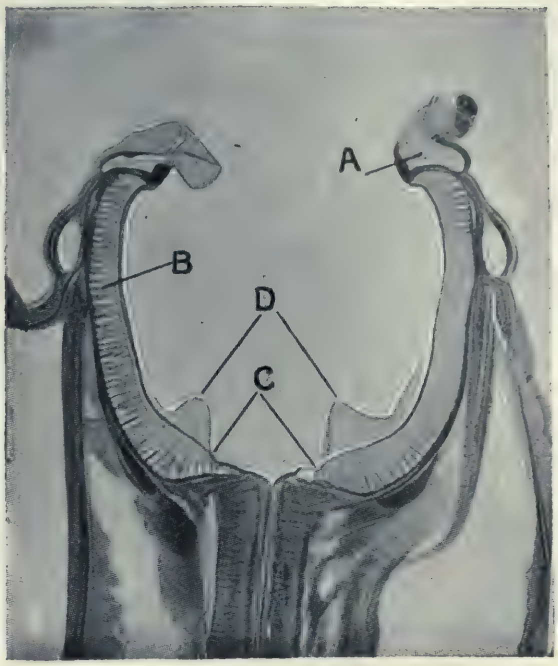

Fig. 6 (× 150). Longitudinal section of the mouth shewing
(A) the membranous lip containing the canal,
(B) the thick chitinous capsule,
(C) elliptical holes in longitudinal section,
(D) chitinous points.

Note the striated appearance of the chitin. This is due to perforations. (See also Fig. 9.)

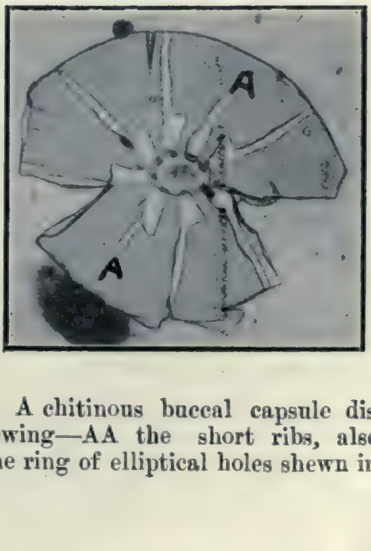

Fig. 7 (× 40). A chitinous buccal capsule dissected out and flattened shewing—AA the short ribs, also the chitinous points and the ring of elliptical holes shewn in Fig. 6.

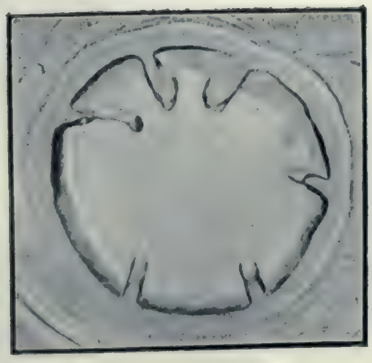

Fig. 8 (× 75). Transverse (almost) section of buccal cavity cut above the point where one pair of ribs disappears leaving six only.

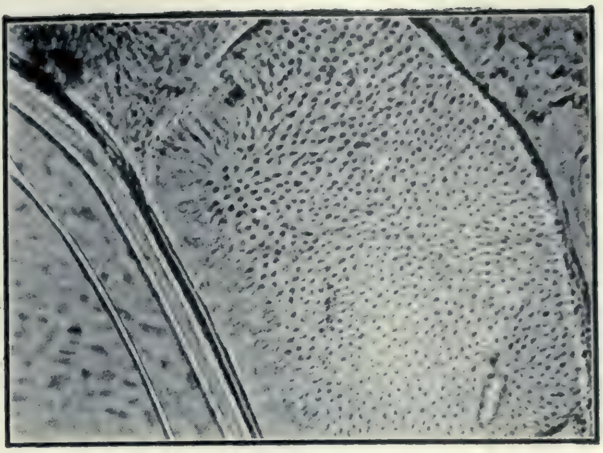

Fig. 9 (× 150). A portion of the buccal capsule seen from the surface. On the left is the membranous lip. The perforations in the chitin are clearly shewn.

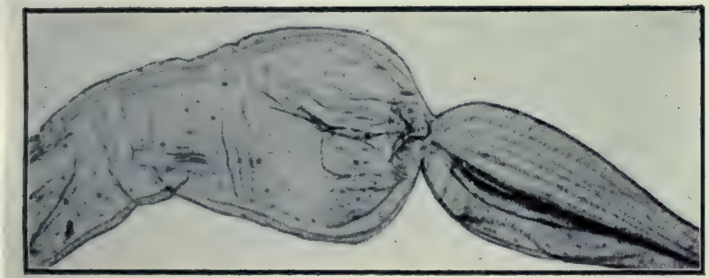

Fig. 10 (× 40). The hinder portion of the œsophagus and the stomach.

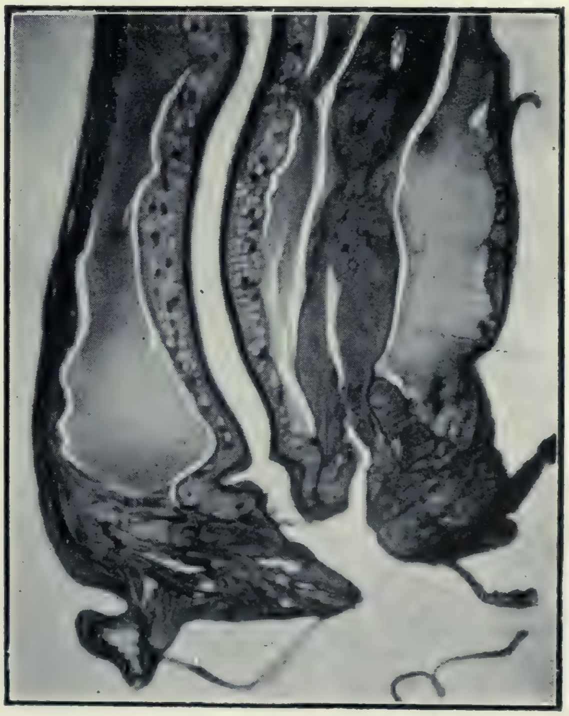

Fig. 12 (× 150). Shewing cloaca with the intestine entering it on the left (*i.e.*, ventrally in the living specimen) and the seminal tube on the right (*i.e.*, dorsally). The terms ventral and dorsal are used assuming that the ventral surface of the male is turned towards the anterior extremity of the female.

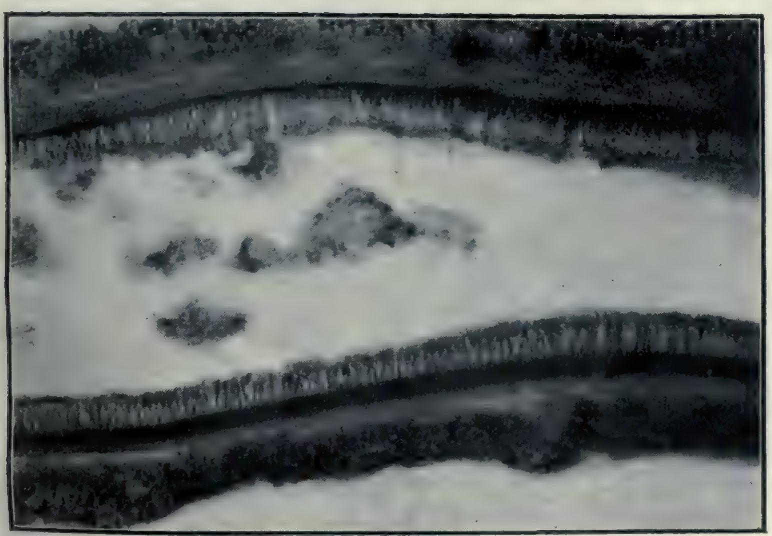

Fig. 11 (× 500). A portion of the intestine shewing the outer cellular layer, the intermediate homogeneous layer, and the inner layer of rods.

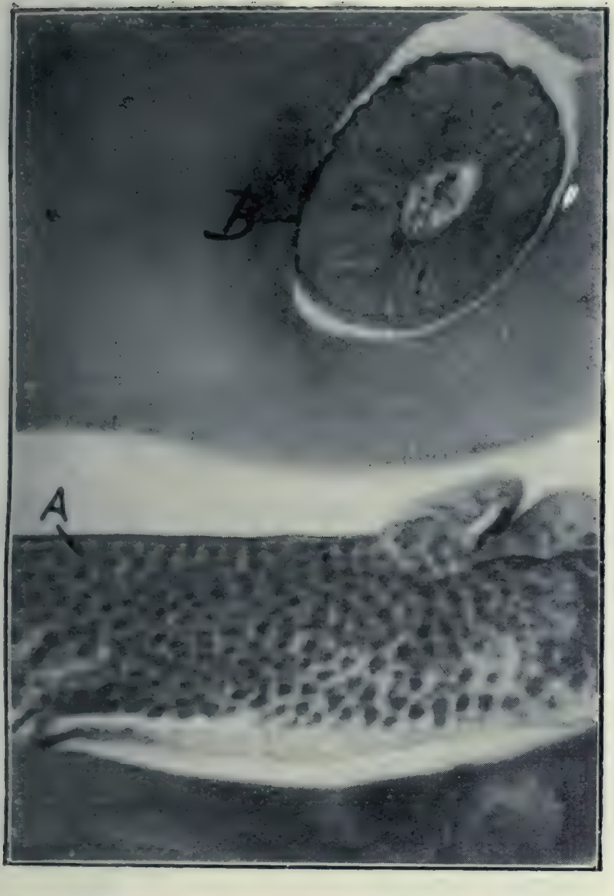

Fig. 13 (×460). Section of two parts of the seminal tube of the male.

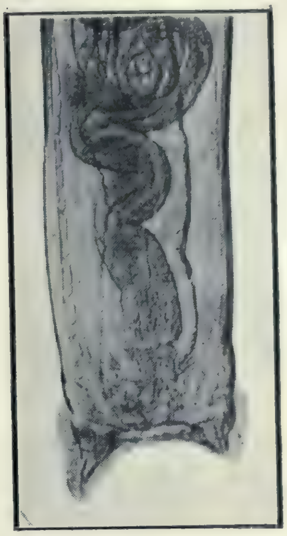

Fig. 14 (×40). Antero-posterior view of the caudal pouch of a detached male (the right half is slightly damaged) shewing the thick fleshy nature and its small size.

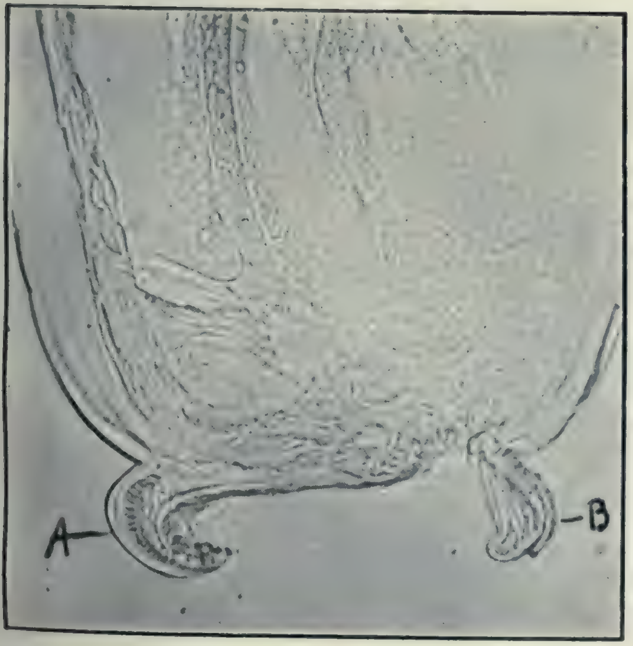

Fig. 15 (×150). Frozen longitudinal section unstained. Shewing at A the recurved anterior flap of the pouch, and at B the less curved posterior flap.

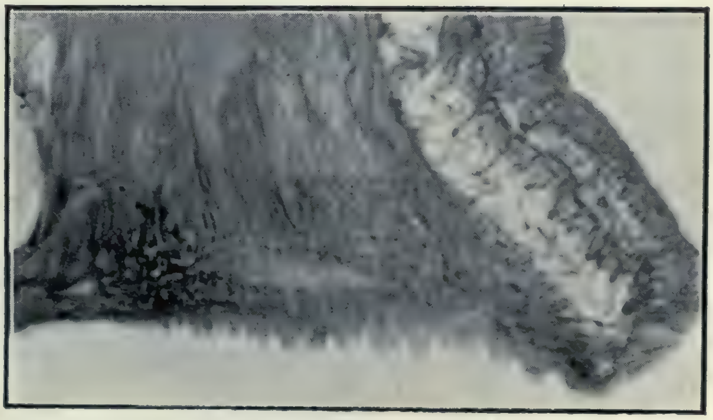

Fig. 16 (×460). Longitudinal section of posterior extremity of male shewing crypts on the inner surface of the pouch.

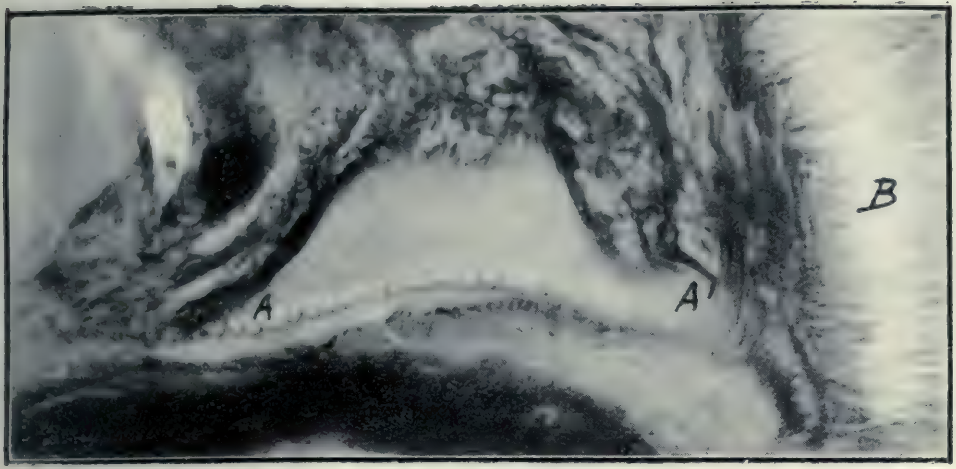

Fig. 17 (× 460). Section through the junction of a male and female. Line of junction at A—A′ shewing the pointed projections fitted into the crypts on the inner surface of the pouch. B, the spongy portion of the pouch.

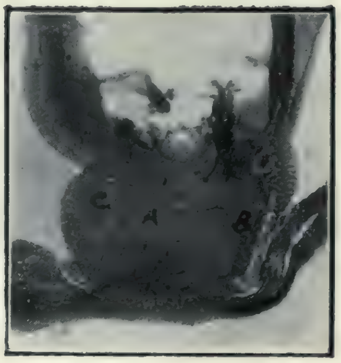

Fig. 18. Longitudinal section through one of the lateral lobes of the pouch shewing the anterior trunk giving rise to three rays, A.
B. Posterior ray.
C. Spongy pouch.

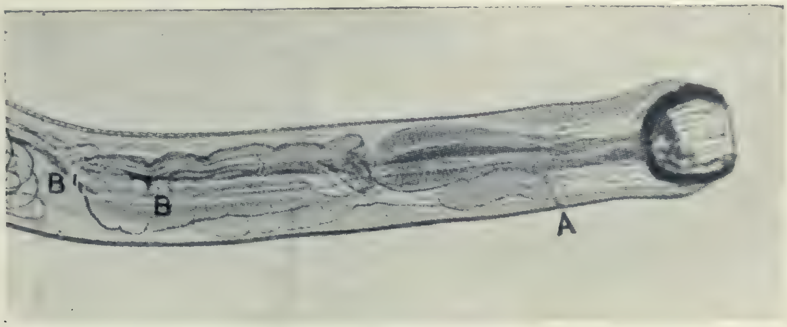

Fig. 19 (× 27). Shewing the two blind pouches extending from the common orifice at A to B & B′. One is partly concealed by the other.

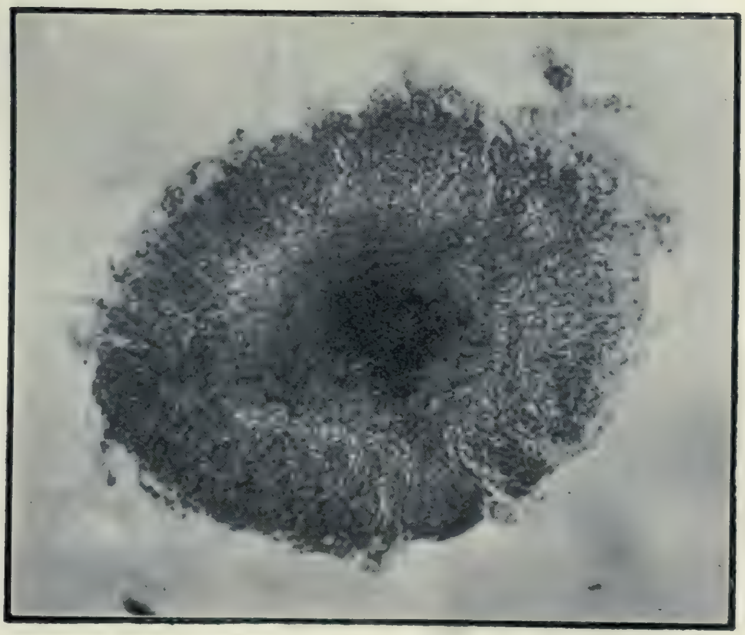

Fig. 20 (× 460). Section of a "rounded" body shewing the other filamentous zone and the inner granular area in which there appear to be some nuclei.

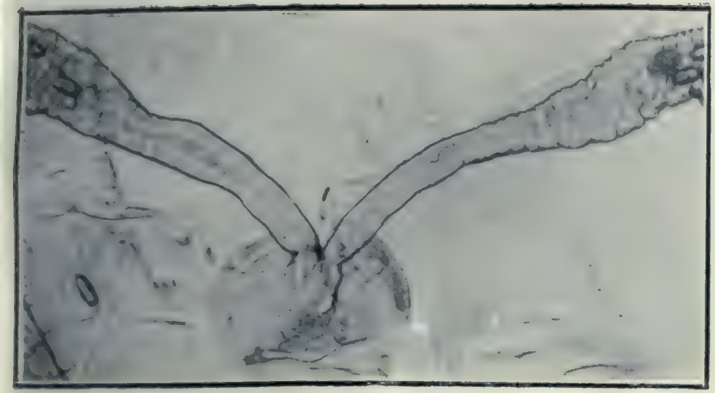

Fig. 21 (× 40). Portion of cuticle of female containing the vulva and shewing the area covered by the pouch of the male, the short vagina, and the anterior portions of the uterine tubes.

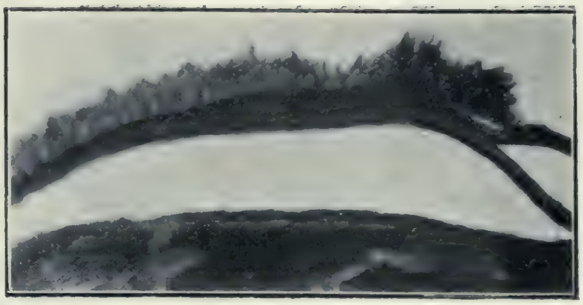

Fig. 22 (× 460). Specialized layer of "boss" of female shewing the projections which fit into the crypts of the pouch of the male.

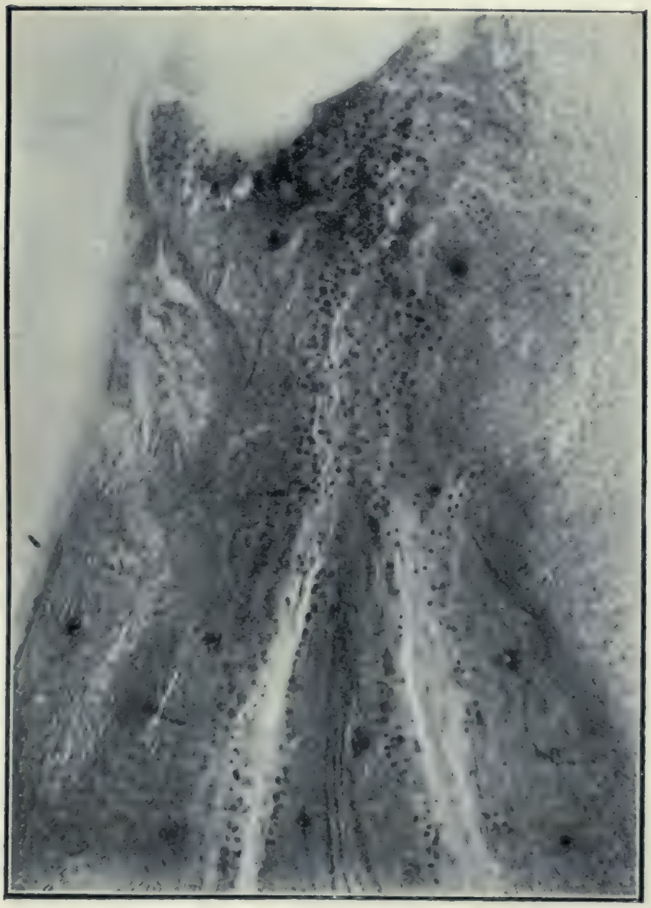

Fig. 23 (× 460). Longitudinal section (dorsi-ventral) of the vulva, short vagina and uterine tubes containing the small cells described in connection with the genital tube of the male.

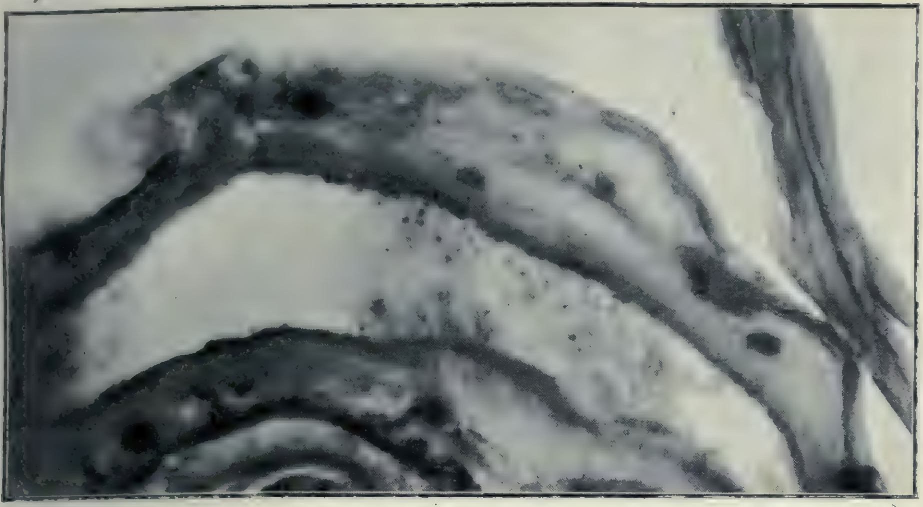

Fig.24(× 460). Oblique section of a portion of the uterus shewing the small cells described in connection with the germinal tube of the male.

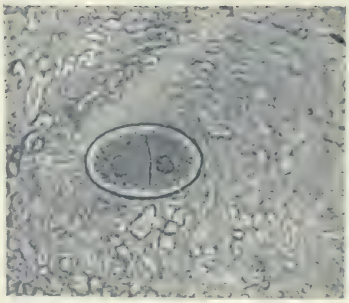

Fig. 26 (× 150). An egg in the mucus from the larynx.

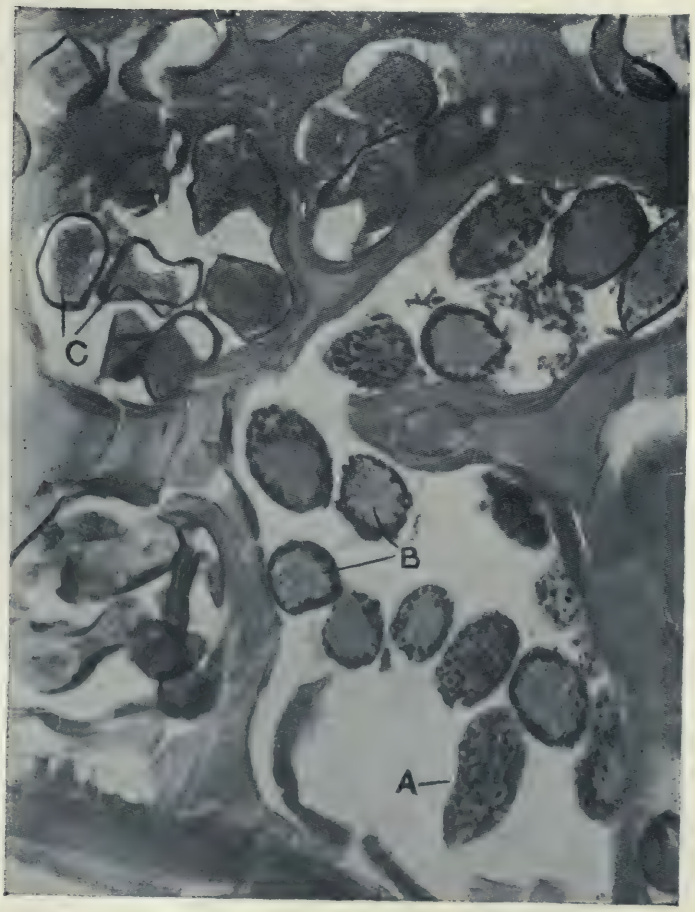

Fig. 25 (× 220). Paraffin section through a female shewing eggs in three stages of development.

A. Eggs crowded with granules.
B. Granules disposed at the margin.
C. Granules disappeared and shell formed (these are deformed by the process of embedding, etc.).

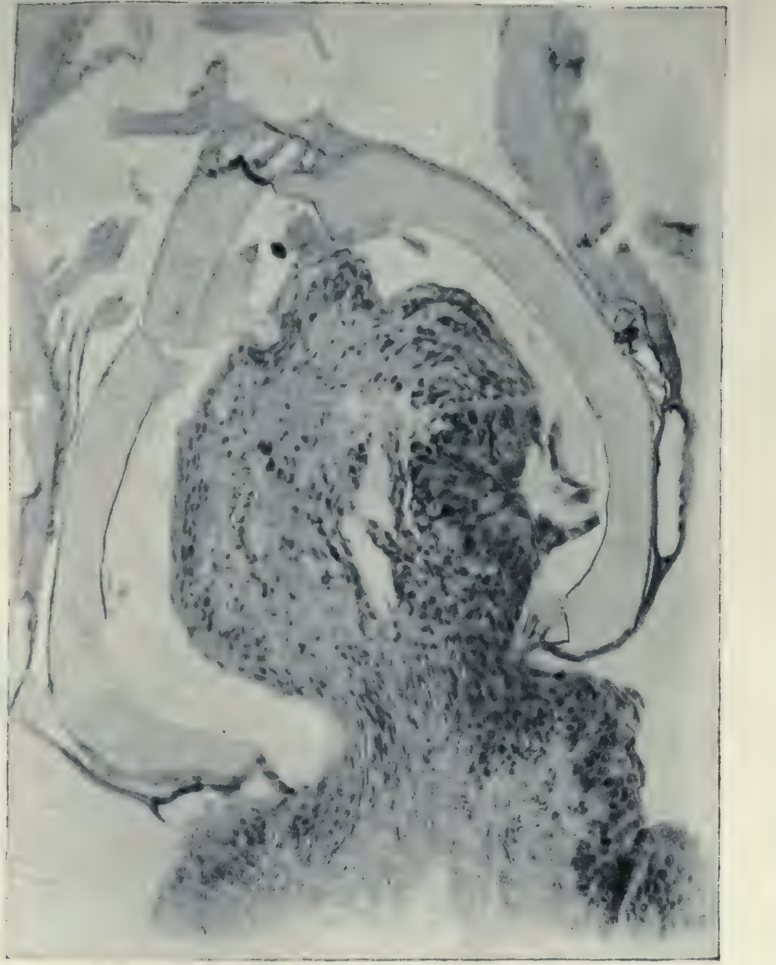

Fig. 27 (×150). Longitudinal section of the mouth of a worm still attached to the mucous membrane, shewing the "bottom" of membrane drawn into the mouth. (In this and the succeeding figure the soft tissues have shrunk somewhat.)

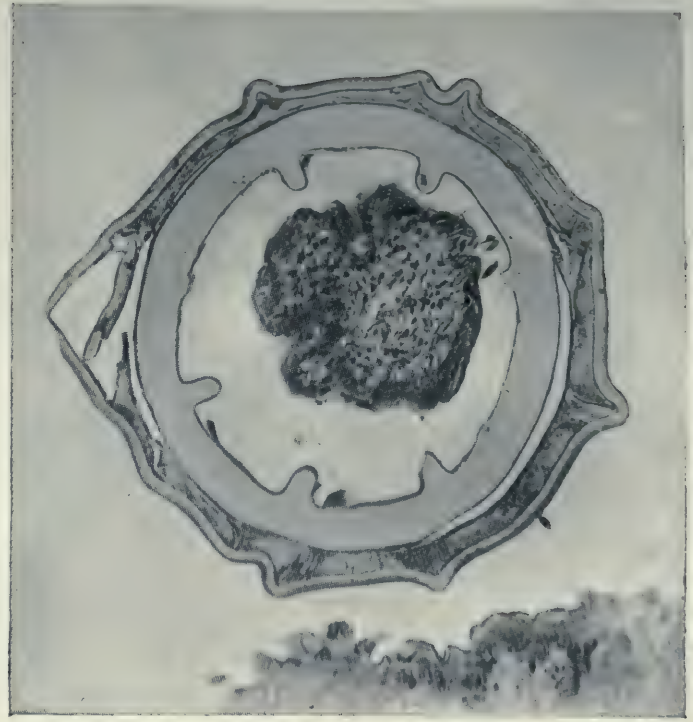

Fig. 28 (×150). Transverse section of the mouth of a parasite still attached to the mucous membrane.

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